ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL EXPRESSION OF P53 IN ORAL SQUAMOUS CELL CARCINOMA, ORAL EPITHELIAL PRECURSOR LESIONS, AND NORMAL ORAL MUCOSA

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ABSTRACT

Objective: To assess the immunohistochemical expression of p53 in tissue samples of oral squamous cell carcinoma (OSCC), oral epithelial precursor lesions, and normal oral mucosa.

Material & Methods: A comparative cross-sectional study was jointly conducted at the Departments of Pathology and Oral and Maxillofacial Surgery of various medical and dental institutes of the country from April 2016 to March 2017. A total of 180 subjects were included in the study. Oral tissue specimens were collected for laboratory investigations after obtaining written consent from all subjects. p53 was assessed using immunohistochemistry in tissue samples of 60 cases of OSCC, 60 cases of epithelial precursor lesions, and normal oral mucosal samples of 60 healthy individuals. Data were recorded, evaluated, and analyzed by SPSS-20.

Results: p53 protein expression was noted in 85% OSCC and 73% oral epithelial precursor lesions. Among healthy individuals, one subject showed p53 immunoreactivity in the normal oral mucosa.

Conclusion: Raised p53 overexpression in OSCC and oral precursor lesions, compared to normal oral mucosa make it a probable candidate for a potential predictive biomarker in oral premalignancy and malignancy.

Keywords: Oral squamous cell carcinoma, Tumor suppressor protein p53, Immunohistochemistry.

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INTRODUCTION

Oral cancer represents a remarkable component of global cancer burden, with raised morbidity and mortality.[1] According to the collective cancer registry report of Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan, from December 1994 to December 2019, the carcinomas of lip and oral cavity is marked as the 3rd most frequently occurring malignant tumor in Pakistan.[2] OSCC is the commonly occurring histopathological variant of oral epithelial malignancy.[3] Frequently, it is anteceded by epithelial precursor lesions. The epithelial precursor lesions are characterized histopathologically as squamous cell hyperplasia with or without other specific cytological and architectural alterations termed as oral epithelial dysplasia (OED), subcategorized as mild, moderate, severe; dysplasia and carcinoma in situ. [4]

OSCC are usually marked by late-stage diagnosis and low survival rates and epithelial precursor lesions are characterized by varied risk of malignant transformation.[3,4] Thus improving the prognosis, researchers are consistently searching for biomarkers that can have a predictive role in clinical practice related to oral malignancy and premalignancy.[5] One of the key events noted in the multistep process of development of oral malignancy and premalignancy is the inactivation of tumor suppres-

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sor genes (TSGs).[6-10] p53 is the most immensely explored gene among the TSGs, linked with oral cancers and associated oral potentially malignant precursors.[9,10] p53 role is noteworthy as in the research literature, it has been titled as "molecule of the year", the guardian of genome & policeman of oncogenes.[11] Epidemiological studies have noted the alterations in p53 gene, leading to accumulation of p53 protein in the tissue samples of oral cancerous and precancerous lesions.[10,12,13,14] In the present study, p53 immunohistochemical status was evaluated among cases of OSCC, OPMDs and healthy individuals to inquire into its clinical usefulness.

MATERIAL & METHODS

The present descriptive cross-sectional study was carried out after approval from the Institutional review board and permissions from the head of the oral and maxillofacial surgical units of Peshawar Dental College (PDC), Khyber college of dentistry (KCD) & PIMS, and head of the histopathology department PIMS, from 3rd April 2016 to 31st March 2017, by adopting a non-probability and purposive sampling technique. The study was conducted on the tissue samples of 180 subjects comprising 60 cases of OSCC (Group A), 60 cases of epithelial precursor lesions (Group B), and 60 healthy individuals (Group C). A detailed history of the study participants was recorded on a structured proforma. Both tobacco users and non-users were part of the study and tobacco usage history was recorded for all the participants (i.e., tobacco usage [Present, Ex, Non-user], type of tobacco products (Smoked tobacco [ST], Smokeless tobacco [SLT], duration of tobacco use in years and daily frequency of tobacco usage). The inclusion criteria for group A includes histopathologically diagnosed cases of OSCC, while for group B includes those oral mucosal biopsies which were characterized by oral epithelial hyperplasia with or without dysplasia and carcinoma in situ. Normal oral mucosa was collected from healthy individuals. The healthy individuals were those who consented to the study participants and visited the recruited centers for dental treatments comprising of 3rd molar surgical extractions, alveoloplasty, etc., in which an extra portion of normal oral mucosal tissue was removed and intended to be discarded due to need of the procedure. [15] The oral mucosal tissues were processed and stained by hematoxylin and eosin for histopathological slide review. The H&E staining confirmed the diagnosis of OSCC and oral epithelial precursor lesions in the tissue samples, while the p53 staining was evaluated by immunohistochemistry by a semi-quantitative scoring system. Special grip-coated slides (Dako Flex IHC Microscope slides) were used for immunohistochemical staining of the tissue samples of the study participants with p53 protein antibody (Clone: DO-7; Antibody type: Monoclonal mouse, Dako, Denmark). The protocol employed for scoring p53 immunoreactivity consists of marking the OSCC and oral epithelial precursor lesion specimen slides either positive or negative. The basic criteria for positive stain were the presence of clear brown nuclear stain. The percentage of stained nuclei was assessed by enumerating p53 stained cells per 100 anaplastic, hyperplastic or dysplastic epithelial cells in the area of best staining with the cut-off value of 10% nuclei stained with p53 immunohistochemically. The p53 stained nuclei counts were categorized into the following 4 categories; absence of the stain or occasional keratinocytes staining (-), staining of 10-33% of keratinocytes (+), staining of 34-66% of keratinocytes (++), staining of greater than 66% of keratinocytes (+++). The intensity of the stain was subjectively graded into the definite but light stain (1+), darker stain (2+), and most intense stain(3+).[12,13] In the tissue sections of the normal oral mucosa, p53 stained nuclei counts were categorized into the following two categories; the negative stain comprise of the absence of expression of p53 protein detected in any epithelial nuclei or even rare cells positive (1-10 cells per section), while the positive p53 immunohistochemical stain was marked when clear brown colored staining with more than 5% of suprabasal cells showed positivity.[14,16,17] In epithelial tissue specimens of oral epithelial precursor lesions and healthy individuals, p53 staining confined exclusively to basal layers only was considered normal expression and marked as a negative case. International Federation of Gynecology and Obstetrics (FIGO) grade 3 endometrioid carcinoma was employed as a positive control for the p53 immunoreactivity.

The data obtained were analyzed by using SPSS version 20. The percentages were calculated for each categorical variable and a Chi-square test was applied for statistical significance, where appropriate. A probability value of less than and equal to 0.05 was considered statistically significant.

RESULTS

The results of our study are summarized in Tables 1-3. The observed mean age of cases of OSCC, epithelial precursor lesions, and healthy individuals was 55 (SD-14.43), 54.5 (SD-14.41), and 50 (SD-11.83) years, respectively.

p53 immunoreactivity was observed in 51(85%) out of 60 lesions of OSCC and 44 (73.3%) out of 60 cases of epithelial precursor lesions. In the normal oral mucosa of healthy individuals, out of sixty samples, only one (1.7%) showed suprabasal staining of p53 protein. A statistically significant difference was recorded among the study participants for p53 immunohistochemical level, staining intensity, and p53 immunoreactivity in tissue specimens of OSCC and OPMD lesions and normal oral mucosa (Table-1).

Among OSCC cases, most of the lesions were WDSCC (46.7%) and among epithelial precursor lesions, most of the lesions presented as squamous cell hyper-

Table 1: p53 immunohistochemical staining level in tissue specimen of the study participants

A) Level of p53 immunohistochemical stain	***OSCC lesions***	***Epithelial precursor lesions***	***Normal oral mucosa of healthy individuals***	***Total***	***p-value***
Absence or occasional keratinocyte staining (-)	9 (15%)	16 (26.7%)	59 (98.3%)	84(46.7%)	<0.01*
Staining of 10-33% of keratinocytes (+)/ Supra basal staining in normal oral mucosa	27 (45%)	40 (66.7%)	1 (1.7%)	68 (37.8%)	
Staining of 33-66% of keratinocytes (++)	10 (16.7%)	4 (6.7%)	-	14 (7.8%)	
Staining of greater than 66% of keratinocytes (+++)	14 (23.3%)	-	-	14 (7.8%)	
B) Staining intensity of p53	***OSCC lesions***	***Epithelial pre-cursor lesions***	***Normal oral mucosa of healthy individuals***	***Total***	***p-value***
					<0.01*
None	9 (15%)	16 (26.7%)	59 (98.3%)	84 (46.7%)	
Definite but light stain (1+)	24 (40%)	20 (33.3%)	1 (1.7%)	45 (25%)	
Darker stain (2+)	23 (38.3%)	19 (31.7%)	-	42 (23.3%)	
Most intense stain (3+)	4 (6.7%)	5 (8.3%)	-	9 (5%)	
C)Tissue p53 Immunoreactivity	***OSCC lesions***	***Epithelial pre-cursor lesions***	***Normal oral mucosa of healthy individuals***	***Total***	***p-value***
Positive	51 (85%)	44 (73.3%)	1 (1.7%)	96 (53.3%)	<0.01*
Negative	9 (15%)	16 (26.6%)	59 (98.3%)	84 (46.6%)	
Total	60 (100%)	60 (100%)	60 (100%)	180 (100%)	

*Pearson's Chi-square test

Table 2: p53 immunoreactivity and histopathological parameters of OSCC and epithelial precursor lesions

Histopathological Features	**Tissue p53 immunoreactivity**		**Total**	**p-value (Chi-square Test)**
	Negative	Positive		
WHO Grading System of OSCC				
Well Differentiated SCC	3(5%)	25(41.7%)	28(46.7%)	0.683*
Moderately Differentiated SCC	5(8.3%)	22(36.7%)	27(45%)	
Poorly Differentiated SCC	1(1.7%)	4(6.7%)	5(8.3%)	
Epithelial precursor lesions				
Squamous cell hyperplasia	8(13.3%)	29(48.3%)	37(61.7%)	0.113*
Mild dysplasia	4(6.7%)	6(10%)	10(16.7%)	
Moderate dysplasia	2(3.3%)	8(13.3%)	10(16.7%)	
Severe dysplasia	-	1(1.7%)	1(1.7%)	
CIS	2(12.5%)	-	2(3.3%)	

*Pearson's Chi-square test

Table 3: Relation between tissue p53 immunoreactivity and age, gender & Tobacco usage (p-value)

Variables	**OSCC**	**OPMDs**	**Healthy Individual**
Age in years >50 <50	0.85b	0.77a	1.0b
Gender Male Female	1.0b	0.21b	0.34b
Tobacco usage Tobacco user Ex tobacco user Non-tobacco user	0.56a	0.27a	0.24b

Type of Tobacco product Smoked tobacco (ST) Smokeless tobacco (SLT) Both ST & SLT	0.29a	0.31a	0.53a
Frequency of tobacco use per day 10-1 times/day >10times/day	0.06b	0.44a	0.06b
Duration of tobacco use in years 10-1 years 20-11 years >20 years	0.38a	0.13a	0.19b

a= Pearson's Chi-square test; b= Fisher's exact test

plasia (N=37/60;61.7%). Statistically, an insignificant difference was observed between p53 immunoreactivity and the WHO histopathological grading system among OSCC and epithelial precursor lesions (Table-2).

Among cases with OSCC lesions, statistically insignificant differences were observed between p53 immunoreactivity status and age, gender, tobacco usage status, type of tobacco product consumed, frequency of tobacco use per day, and in years (Table-3).

Among healthy individuals, out of 60 tissue specimens of the normal oral mucosa, only one (1.7%) sample showed suprabasal staining of p53 protein (Table-1).

DISCUSSION

Oral squamous cell carcinoma (OSCC) is the frequently occurring oral epithelial malignancy that mostly originates from oral potentially malignant disorders. [4] There is always a perpetual search for a biomarker that can assist in the timely prediction of malignant transformation of epithelial precursor lesions for improving the prognosis of OSCC.[5] In the present study, tissue p53 immunoreactivity was assessed in OSCC and epithelial precursor lesions with taking in consideration the normal oral mucosa to unfold its possible predictive role in the timely indication of oral malignancy and premalignancy.

The present study reported that most of the patients with OSCC and epithelial precursor lesions presented in the same mean age of 50±years as reported by other researchers.[3,18,19] Old age preponderance among cases of oral malignant and premalignant lesions is in obedience with the observations noted by other researchers also, who have marked age as a fear factor in OSCC development and foretelling index in potentially malignant epithelial precursors.[20-22]

In the present study, the highest frequency of tissue p53 immunoreactivity was noted among lesions of OSCC (85%) followed by epithelial precursor lesions (73%) compared to the normal oral mucosa (1.7%).

The observation of the highest percentage of p53 tissue expression among lesions of OSCC is comparable to studies in the region that reported p53 immunoexpression in a varied range. Ara et al., Hashmi et al., and Ghanghoria et al. reported tissue p53 phosphoprotein expression with a frequency of 67%, 66.1%, 63% and 54%.[23,18,24,25]

The difference in p53 protein expression may be due to the difference in p53 antibody clone used or regional risk habits related to OSCC lesions development.[8,25]

The present study observed a statistically significant relation between p53 staining intensity and p53 protein expression in tissue samples of OSCC. These findings are in obedience with the findings noted by Ara et al., and Azizi et al.,[23,26]

Among OSCC lesions, a statistically insignificant relation was observed between tissue p53 immunoreactivity and WHO OSCC grading system, age, gender, tobacco usage history, type of tobacco product consumed frequency of tobacco use per day, and duration of tobacco use in years. These findings are in obedience with the observations reported by Bhattacharya et al. [27] However, a study done by Gatto et al., revealed a statistically significant relation between p53 immunoreactivity and histological grades and tobacco and betel quid habits and insignificant relation with age, gender of OSCC lesion.[28]

The present study noted that among epithelial precursor lesions, 73.3% (N=44/60) showed tissue p53 immunoreactivity. This finding is comparable to the reported frequency of p53 immunoexpression in local and international studies.[21,29,30]

The present study recorded a statistically significant (p=<0.01) relation between p53 staining intensity in tissue and p53 expression among cases with epithelial precursor lesions, contrary to the study by Nagata et al., who reported a statistically insignificant difference between p53 staining intensity and p53 expression among cases of oral epithelial precursors.[31]

The present study noted an insignificant relation of p53 immunoreactivity with age, gender, tobacco usage history, type of tobacco product consumed, frequency of tobacco use per day, and duration of tobacco use in years, among cases with oral epithelial precursor lesions. These findings are consistent with a study conducted by Nagata et al., 2018; who recorded a statistically insignificant relation between p53 immunoreactivity and age and gender.[31]

Among healthy individuals, regarding normal oral mucosa, the present study revealed that only one subject

expressed p53 immuno-staining in suprabasal layers. These findings are contrary to other studies that observed that p53 protein expression was exclusively absent in all oral epithelial layers or present in the basal layer only but not noted in the suprabasal layers.[28,29]

Detection of p53 protein in normal oral mucosa is mostly absent due to the brief half-life of the wild type of p53 protein or due to expression of minimal quantity, which is difficult to be detected on immunohistochemistry.[32] The possible explanation for the collection of wild type of p53 phosphoprotein is that it might be an outcome of the defect in the degradation pathway or binding of wild type proteins to other proteins leading to the gathering of stabilized normal proteins or nonfunctional p53 phosphoproteins or probably as a physiological response of cells to the genotoxic stress.[19, 24] Among healthy individuals, the present study observed that p53 immunoreactivity was not significantly related to the age, gender, tobacco use status, type of tobacco products, tobacco use frequency per day, and duration of tobacco use in years.

CONCLUSION

The present study observed an increase in p53 protein expression in OSCC as compared to oral precursor lesions and decreased expression in the normal oral mucosa. Thus, concluding that p53 immunoreactivity can probably predict the susceptibility of potentially malignant tissue to transform into oral malignancy.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Khan AS:	Concept/ Idea, Literature, review, Drafting & Final Review
Ahmed S:	Concept/ Idea, Analysis & Interpretation of Data, References
Iqbal F:	Analysis & Interpretation of Data
Saboor A:	Manuscript Writing, Literature review, Analysis & Interpretation of Data
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Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.